

Ability of Standard Toxicity Tests to  
Predict the Effects of the Insecticide  
Diflubenzuron on Laboratory Stream Communities

(U.S.) Corvallis Environmental Research Lab., OR

1 Jun 82



U.S. Department of Commerce  
National Technical Information Service

**NTIS**

| TECHNICAL REPORT DATA<br>(Please read Instructions on the reverse before completing)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                  |                                                          |                                          |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|----------------------------------------------------------|------------------------------------------|
| 1. REPORT NO.<br>EPA-600/J-82-264                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 2. JOURNAL ARTICLE                               | 3. R                                                     | PB83-157560                              |
| 4. TITLE AND SUBTITLE<br>JOURNAL ARTICLE:<br>Ability of Standard Toxicity Tests to Predict the Effects of the Insecticide Diflubenzuron on Laboratory Stream Communities                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                  | 5. REPORT DATE<br>1982                                   | 6. PERFORMING ORGANIZATION CODE          |
| 7. AUTHOR(S)<br>S.R. Hansen and R.R. Garton                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                  | 8. PERFORMING ORGANIZATION REPORT NO.                    |                                          |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS<br>Environmental Research Laboratory<br>U.S. Environmental Protection Agency<br>Corvallis, Oregon 97333                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                  | 10. PROGRAM ELEMENT NO.                                  | 11. CONTRACT/GRANT NO.                   |
| 12. SPONSORING AGENCY NAME AND ADDRESS<br>same<br><i>The authors</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                  | 13. TYPE OF REPORT AND PERIOD COVERED<br>Journal article | 14. SPONSORING AGENCY CODE<br>EPA/600/02 |
| 15. SUPPLEMENTARY NOTES<br>Published in : Canadian Journal of Fisheries and Aquatic Sciences<br>Vol. 39, no. 9, 1982, pp 1273-1288                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                  |                                                          |                                          |
| 16. ABSTRACT<br>We assessed the ability of a standard set of freshwater single species toxicity test to predict accurately effects of the insecticide diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea) on complex laboratory stream communities. The single-species tests complied with requirements prescribed for establishing freshwater quality criteria and included nine freshwater animal acute tests, five freshwater animal chronic tests, and one freshwater algal test. The stream communities were stocked from a natural source, equilibrated for 3 mo and then treated with diflubenzuron for 5 mo. Effects on these stream communities were assessed at the functional group level using biomass and diversity for the analysis. The single-species tests adequately predicted the concentrations of diflubenzuron which affected these stream communities; the most sensitive test species, insects and crustaceans were up to an order of magnitude more sensitive than the observed community effects. The single-species tests were less successful in predicting the exact nature of the community level effects. Those effects resulting from direct lethality to component species were clearly predicted; indirect effects due to altered interspecies interactions could only be predicted with an a priori knowledge of the system's trophic dynamics. |                                                  |                                                          |                                          |
| 17. KEY WORDS AND DOCUMENT ANALYSIS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                  |                                                          |                                          |
| a. DESCRIPTORS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | b. IDENTIFIERS/OPEN ENDED TERMS                  | c. COSATI Field/Group                                    |                                          |
| REPRODUCED BY<br><b>NATIONAL TECHNICAL<br/>INFORMATION SERVICE</b><br>U.S. DEPARTMENT OF COMMERCE<br>SPRINGFIELD, VA 22161                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                  |                                                          |                                          |
| 18. DISTRIBUTION STATEMENT<br>Release to public                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 19. SECURITY CLASS (This Report)<br>Unclassified | 21. NO. OF PAGES<br>17                                   |                                          |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 20. SECURITY CLASS (This page)<br>Unclassified   | 22. PRICE                                                |                                          |

JOURNAL ARTICLE:

**Ability of Standard Toxicity Tests to Predict the Effects of the Insecticide  
Diflubenzuron on Laboratory Stream Communities**

STEPHEN R. HANSEN<sup>1</sup> AND RONALD R. GARTON

*U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, Corvallis, OR 97333, USA*

HANSEN, S. R., AND R. R. GARTON. 1982. Ability of standard toxicity tests to predict the effects of the insecticide diflubenzuron on laboratory stream communities. *Can. J. Fish. Aquat. Sci.* 39: 1273-1288.

We assessed the ability of a standard set of freshwater single-species toxicity tests to predict accurately effects of the insecticide diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea) on complex laboratory stream communities. The single-species tests complied with requirements prescribed for establishing freshwater quality criteria and included nine freshwater animal acute tests, five freshwater animal chronic tests, and one freshwater algal test. The stream communities were stocked from a natural source, equilibrated for 3 mo and then treated with diflubenzuron for 5 mo. Effects on these stream communities were assessed at the functional group level using biomass and diversity for the analysis. The single-species tests adequately predicted the concentrations of diflubenzuron which affected these stream communities; the most-sensitive test species, insects and crustaceans, were up to an order of magnitude more sensitive than the observed community effects. The single-species tests were less successful in predicting the exact nature of the community level effects. Those effects resulting from direct lethality to component species were clearly predicted; indirect effects due to altered interspecies interactions could only be predicted with an *a priori* knowledge of the system's trophic dynamics.

**Key words:** toxicity, diflubenzuron, streams, community effects, toxicity testing

HANSEN, S. R., AND R. R. GARTON. 1982. Ability of standard toxicity tests to predict the effects of the insecticide diflubenzuron on laboratory stream communities. *Can. J. Fish. Aquat. Sci.* 39: 1273-1288.

Nous avons évalué la capacité d'une série d'essais de toxicité standard avec espèces uniques à prédire correctement les effets de l'insecticide diflubenzuron (1-(4-chlorophényl)-3-(2,6-difluorobenzoyl)urée) sur des communautés fluviales complexes en laboratoire. Ces essais sont conformes aux normes de définition des critères de qualité de l'eau douce et comportent neuf essais aigus, cinq essais chroniques et un essai avec algues, tous organismes dulçaquicoles. Les communautés, provenant de source naturelle, ont été équilibrées pendant 3 mois et ensuite traitées au diflubenzuron pendant 5 mois. Nous avons évalué les effets sur ces communautés au niveau de groupe fonctionnel, en fondant l'analyse sur la biomasse et la diversité. Grâce à ces essais, on peut prédire convenablement les concentrations de diflubenzuron qui influencent ces communautés; les effets sont plus prononcés sur les espèces les plus sensibles, insectes et crustacés, que sur la communauté, et ce d'un facteur allant jusqu'à un. Par ailleurs, les essais avec espèces uniques ne permettent pas de prédire avec autant de succès la nature exacte des effets au niveau de la communauté. Les effets résultant d'une létalité directe à l'endroit des espèces constituantes sont clairement prédits; les effets indirects découlant de modifications des interactions entre espèces ne peuvent être prédits qu'avec une connaissance *a priori* de la dynamique trophique du système.

Received February 4, 1981  
Accepted June 1, 1982

Reçu le 4 février 1981  
Accepté le 1 juin 1982

THE normal functioning of a freshwater aquatic biological community is in part a result of its species composition and the state of the interactions between these component species.

These two factors are coupled in a complex manner, not easily modeled or quantified. When toxic substances enter natural aquatic systems, both underlying factors can be affected, resulting in altered community structure and function.

Currently, in the development of freshwater quality criteria, the potential of a substance to harm natural aquatic communities is assessed primarily by examining direct toxic effects on a prescribed set of single-species populations, using

<sup>1</sup>Present address: Ecological Analysts, Inc., 2150 John Glenn Drive, Suite 100, Concord, CA 94520, USA.

methods published in the United States Federal Register on March 15, 1979 (44FR15926), July 25, 1979 (44FR43660), and October 1, 1979 (44FR56628). In these tests, organisms are exposed to the suspected toxicant and alterations in population level parameters (i.e. mortality, fecundity, and growth rate) are used as the effect criteria. Indirect toxic effects due to altered interspecies or intraspecies interactions are not considered.

Because of the urgency of water pollution problems, the use of these single-species tests has gained wide acceptance. They are simple, provide rapid generation of data, and ecological significance of the results is apparent. However, considerable uncertainty has been expressed concerning the ability of these single-species tests to predict accurately actual effects which would be experienced in natural complex biological communities (Mount 1979, 1981; Cairns 1980; Gibson 1981; Reisa 1981). This uncertainty arises because of a number of basic assumptions which are inherent in the sole use of these tests in setting water quality standards. First, it is assumed that the standard test species exhibit a range of sensitivities sufficient to protect most, if not all, species in natural assemblages. Second, it is assumed that interactions between species need not be considered separately. Either these interspecies interactions are adequately protected by single-species test results (i.e. the sensitivity of the interactions is equal to, or less than, the sensitivity of the single-species population parameters) or, if the interactions are more sensitive, the alterations in these interactions have little impact on the function and structure of the community. It has been suggested that the use of more complex test systems may reduce these deficiencies associated with single-species tests, and therefore, produce more predictive results (Heath 1979; U.S.E.P.A. 1981; N.R.C. 1981). These proposed complex systems offer the advantages of simultaneously testing large numbers of species and including interactions between species in their assessment of toxicity. However, because of high cost, slow generation of data, and ambiguous ecological significance of results, their use in criteria development has been retarded and reliance on single-species tests continues.

The ability of these standard single-species toxicity tests to predict aquatic community level effects has received only limited direct experimental confirmation. In a few studies, for a limited number of toxic substances, comparisons have been made between the results generated in standard single-species tests and in laboratory or field community level systems (Eisele and Hartung 1976; Hitchcock 1965; Geckler 1976; Weiss 1976). In each case, the results generally support the adequacy of single-species tests. Concentrations of toxicants that cause community level effects are within an order of magnitude of those concentrations predicted as harmful by the standard bioassays. However, the nature of the community level effects was often not well predicted. None of these studies are critical tests of the predictive ability of the single-species tests. The studies either do not include the complete prescribed set of test species, and/or they fail to monitor sufficiently the structure and function of the entire biological community.

The objective of this study was to examine more critically the predictive ability of the single-species tests and, therefore, to test the validity of their underlying assumptions. The strat-

egy was to determine effect levels of the insecticide diflubenzuron (1-(4-chlorophenyl)-3-(2,6 difluorobenzoyl)urea), a chitin synthesis inhibitor, on a standard set of freshwater test organisms and to compare the results with effects observed in complex laboratory stream communities. Comparisons of effect concentrations and the nature of such effects were then used to assess predictive ability.

## Materials and Methods

These studies were conducted at the U.S. Environmental Protection Agency's Corvallis Environmental Research Laboratory in Corvallis, OR. The duration of the stream study was ~9 mo, August 1979–May 1980. The single-species toxicity tests were performed June–September 1980.

### STANDARD SINGLE-SPECIES TESTS

In this study, the set of single-species toxicity tests was chosen according to the guidelines established for deriving freshwater quality criteria, as published in the U.S. Federal Register on March 15, 1979 (44FR15970). These guidelines require the use of at least eight animal acute tests, three animal chronic tests, and one freshwater algal test. The acute toxicity tests must incorporate freshwater animal species representing eight different families, including at least one salmonid fish, one non-salmonid fish, one planktonic crustacean, one benthic crustacean, one benthic insect, and one benthic insect detritivore. The chronic tests must incorporate three aquatic animal species, including at least one fish, one invertebrate, and at least one of the three must be a freshwater species.

The actual set of single-species toxicity tests used in this study met these guideline requirements and included nine animal acute tests (Table 1), five animal chronic tests, and one freshwater algal test (Table 2). These tests employed standard exposure and analysis methods (ASTM 1980; U.S.E.P.A. 1975; Nebeker 1973; Garton 1980; Andros and Garton 1980); more detailed descriptions of the methods and results are not yet published (A. Nebeker and G. Chapman, U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR). Where applicable, life stages were chosen so that a molt would occur during the test period, thus ensuring the inclusion of effects on chitin synthesis in the toxicity assessment.

The determinations of effects in these single-species tests were made at the population level. The parameters of analysis are survival in the acute tests and survival, fecundity, and growth in the chronic tests. Reductions in these parameters are assumed to indicate direct toxic effects.

The maximum concentration of diflubenzuron used in these tests varied between 50 and 90 µg/L, resulting from changes in experimental conditions (e.g. water temperature) during the course of the study. Analysis of the concentration of diflubenzuron in the water of each test system was performed throughout the test period by high-pressure liquid chromatography, using a single-band 254-nm UV detector, a micro-Bondapak C-18 column, and a 60:40 acetonitrile–water mobile phase. Because of the sensitivity limit of the analysis procedure, reported concentrations  $\geq 1.0$  µg/L are measured

TABLE 1. Acute toxicity tests using insecticide diflubenzuron.

| Factors                                           | Steelhead trout ( <i>Salmo gairdneri</i> ) | Fathead minnow ( <i>Pimephales promelas</i> ) | Guppy ( <i>Lebistes reticulatus</i> ) | Midge ( <i>Cricotopus</i> sp.) | Midge ( <i>Tanytarsus dissimilis</i> ) | Gammarid ( <i>Hyallela azteca</i> ) | Daphnia ( <i>Daphnia magna</i> ) | Snail ( <i>Juga plicifera</i> ) | Snail ( <i>Physa</i> sp.) |
|---------------------------------------------------|--------------------------------------------|-----------------------------------------------|---------------------------------------|--------------------------------|----------------------------------------|-------------------------------------|----------------------------------|---------------------------------|---------------------------|
| Class of test species                             | Salmonid fish                              | Non-salmonid fish                             | Non-salmonid fish                     | Benthic insect                 | Benthic detritivore insect             | Benthic crustacea                   | Planktonic crustacea             | Freshwater mollusk              | Freshwater mollusk        |
| Initial age of organisms                          | 5 mo                                       | 2 mo                                          | 30 d                                  | —                              | —                                      | —                                   | 24 h                             | —                               | 1.5 mo                    |
| Initial size or developmental stage               | 58 mm                                      | 3.5 mm                                        | —                                     | 4th instar                     | 2nd instar                             | 2-4 mm                              | —                                | 3-6 mm                          | 12-20 mm                  |
| Test duration                                     | 96 h                                       | 96 h                                          | 96 h                                  | 7 d                            | 5 d                                    | 5 d                                 | 48 h                             | 96 h                            | 7 d                       |
| Flow type                                         | Flow through                               | Flow through                                  | Flow through                          | Flow through                   | Flow through                           | Flow through                        | Static                           | Flow through                    | Flow through              |
| Criteria for effect determination                 | Survival LC50                              | Survival LC50                                 | Survival LC50                         | Molting success                | Molting success                        | Survival LC50                       | Survival LC50                    | Survival LC50                   | Survival LC50             |
| Water temp. (°C)                                  | 11                                         | 20                                            | 20                                    | 20                             | 20                                     | 20                                  | 20                               | 20                              | 20                        |
| Water hardness (mg/L as CaCO <sub>3</sub> equiv.) | 24                                         | 25                                            | 25                                    | 25                             | 25                                     | 25                                  | 50                               | 25                              | 25                        |

TABLE 2. Chronic toxicity tests using insecticide diflubenzuron.

| Factors                                                 | Steelhead trout<br>( <i>Salmo gairdneri</i> )                             | Fathead<br>minnow<br>( <i>Pimephales<br/>promelas</i> ) | Daphnia<br>( <i>Daphnia magna</i> ) | Algae<br>( <i>Selenastrum<br/>capricornutum</i> ) | Snail<br>( <i>Juga<br/>plicifera</i> ) | Snail<br>( <i>Physa</i> sp.)         |
|---------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------|-------------------------------------|---------------------------------------------------|----------------------------------------|--------------------------------------|
| Class of<br>test species                                | Salmonid fish                                                             | Non-salmonid<br>fish                                    | Planktonic<br>crustacea             | Freshwater<br>algae                               | Freshwater<br>mollusk                  | Freshwater<br>mollusk                |
| Initial age<br>of organisms                             | 5 h postegg<br>fertilization                                              | Egg                                                     | 24 h                                | Exponential<br>growth                             | —                                      | 1.5 m                                |
| Initial<br>size or<br>developmental<br>stage            | —                                                                         | —                                                       | —                                   | —                                                 | 3–6 mm                                 | 12–20 mm                             |
| Test<br>duration                                        | 9 wk<br>(including 6 wk<br>posthatch<br>exposure with<br>4 wk of feeding) | 30 d                                                    | 21 d                                | 120 d                                             | 2 wk                                   | 2 wk                                 |
| Flow type                                               | Flow through                                                              | Flow through                                            | Static                              | Static                                            | Flow through                           | Flow through                         |
| Criteria<br>for effect<br>determination                 | Survival,<br>growth                                                       | Survival,<br>growth                                     | Survival,<br>reproduction           | Population,<br>growth (dry<br>weight)             | Survival,<br>growth                    | Survival,<br>growth,<br>reproduction |
| Water<br>temp. (°C)                                     | 11                                                                        | 20                                                      | 20                                  | 24                                                | 20                                     | 20                                   |
| Water hardness<br>(mg/L as<br>CaCO <sub>3</sub> equiv.) | 25                                                                        | 25                                                      | 50                                  | —                                                 | 25                                     | 25                                   |

values, whereas those concentrations  $<1.0 \mu\text{g/L}$  are nominal values (Corley et al. 1974; Oehler and Holman 1975).

#### COMPLEX LABORATORY STREAM COMMUNITY TESTS

Community level studies were carried out in 10 indoor continuous-flow laboratory stream channels (Fig. 1). Rocks, 7.5–13.0 cm in diameter, were added to the riffle portions of each channel to a depth of ~15 cm; no rocks were placed in the pool areas. Well water (see Samuelson 1976 for a detailed history of water quality) was added continually at a rate of 5 L/min and maintained at a depth of 10 cm above the rocks by means of standpipes, establishing a 95% replacement rate of 4.5 h. A midstream surface current of 30 cm/s was maintained by using stainless steel, motordriven paddle wheels. A uniform light intensity of  $2.2 \times 10^3 \text{ lx}$  (200 ft. candles) was maintained over each channel, using suspended metal halide lamps (Maxi-Gro, 1000 W. Sylvania GTE Agronomics, Waltham, MA.<sup>2</sup>) A natural diurnal light cycle was set with 30-min dawn-and-dusk low light periods.

The fauna were introduced into the stream channels through the replacement water supply and via field collections. Introductions via the water inflow were continuous, but

limited to microorganisms (i.e. primarily algae and bacteria). Periodic field collections supplied more diverse fauna. During August–October 1979, biological collections were made twice weekly from Rock Creek, a second-order forest stream in the Mary's River watershed, 16 km west of Corvallis, OR. A combination of kicking and rock-brushing techniques dislodged organisms and facilitated net collections. After removing fish and crayfish, the field samples were randomly distributed among the 10 stream channels. Fish and crayfish were eliminated to maintain the viability of the insect fauna of these stream communities. The large energy demands of even a few of these predators coupled with the lack of insect immigration into these laboratory systems would quickly devastate the insect populations and prevent longterm assessments of diflubenzuron impact on this group.

Detrital material, essential as a food source for the stream decomposers, was manually introduced into the channels. In the field collections, varying amounts of leafy and woody material were also trapped. This detritus was distributed among the channels, adding an estimated 50–100 g of allochthonous material per stream per sampling event. After this initial stocking, monthly supplements of 50 g of freezer-preserved dead alder (*Alnus* sp.) leaves were added to each channel.

In all 10 laboratory channels, the biota were allowed to develop and equilibrate for 3 mo (September–November

<sup>2</sup>Mention of product does not constitute endorsement by the U.S. Environmental Protection Agency.

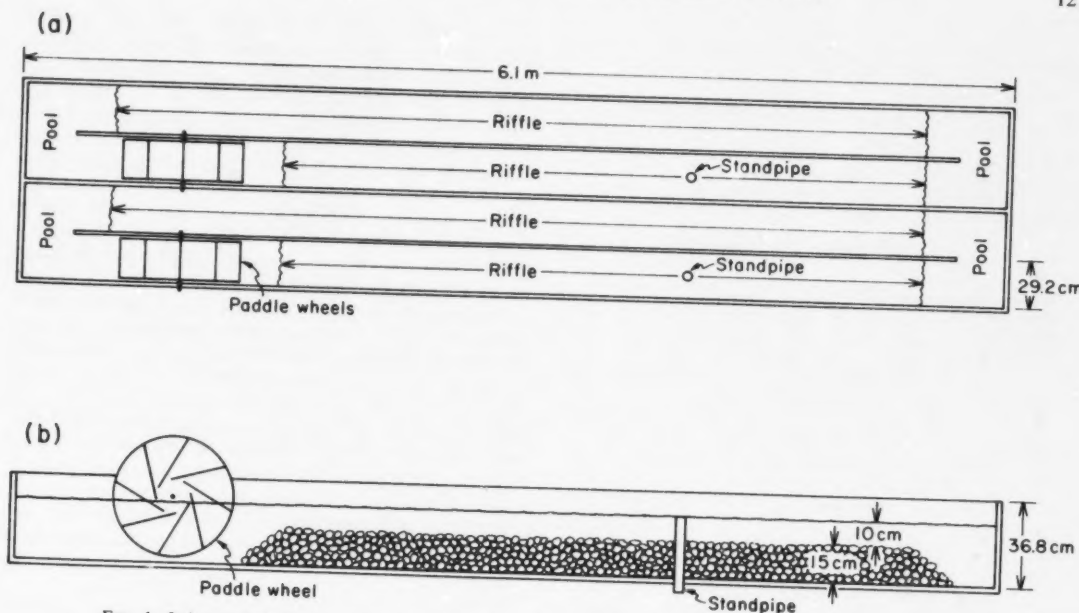


FIG. 1. Laboratory stream channels: (a) an overview of a pair of channels; (b) a side view of a single channel.

1979). Equilibration involved the establishment, in all streams, of similar diversity and biomass for each of the component functional groups (Fig. 3–9). The channels were then separated into five pairs, and diflubenzuron treatments were carried out for the following 5 mo (December 1979–May 1980). Four stream pairs received continuous addition of diflubenzuron; each pair received one concentration of either 0.1, 1.0, 10, or 50  $\mu\text{g/L}$ . Dimethylformamide (DMF) served as the carrier solvent and was maintained at 1 mg/L in all treatment streams. The fifth stream pair served as carrier controls, with continuous addition of 1 mg/L of DMF. The chemical characteristics of the water in the 10 stream channels were monitored throughout the study. Water temperature was measured continuously; diflubenzuron concentration, pH, D.O., hardness, and alkalinity weekly; and monthly measurements were taken of nitrate, nitrite, ammonia, organic nitrogen, and orthophosphate.

The biota in each stream were estimated monthly throughout the October 1979–May 1980 study period. Three samples were taken from each channel; each sample was collected by sealing off a 0.052-m<sup>2</sup> area, dislodging attached organisms, and siphoning out the enclosed water and suspended organisms. Samples were divided for analyses of the composition and biomass of the invertebrate, algal, fungal, and bacterial components.

The composition of the invertebrate fauna was estimated by filtering the stream samples through a Tyler 115-mesh metal screen and identifying the collected organisms. Insects other than the dipteran family Chironomidae were identified to at least the genus level. The Chironomidae were identified to family, subfamily, or tribe.

The biomass of each invertebrate functional group was estimated by the total cumulative length of all sampled

organisms taxonomically assigned to that group. The use of these cumulative lengths as biomass estimates are, undoubtedly, approximations; however, we feel they are adequate for these between-stream comparisons. Because of differences in geometry between the genera comprising each functional group, the use of genus-specific weight/length regressions would have been preferable in producing the most accurate estimates. Unfortunately, such regressions do not exist for a large number of the genera in question. However, preliminary data generated from other very similar stream communities (C. Callahan, U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR., personal communication) support the validity of our approximation. Regressions of the total cumulative weight of mixed genus invertebrate samples against the cumulative length of such samples produced results for invertebrates that were statistically significant at the 95% level of confidence.

The composition of the diatom flora, which represented ~80% of all algae in the streams, was estimated from slide preparations. Each sample was blended and an aliquot evaporated onto a glass slide. The organic matter was burned off, and the remaining silica valves were mounted in Hyrax. Microscopic identifications (1000 $\times$ , oil immersion) to at least the genus level of the first 100 randomly encountered cells were used to estimate relative abundances in each channel and to calculate diversity.

An estimate of algal biomass was made from the determination of the chlorophyll *a*, *b*, *c*, and pheophytin concentrations in each stream channel. Each sample was blended, treated with  $\text{MgCO}_3$ , and centrifuged. The precipitate was dissolved in 90% acetone, sonicated, and centrifuged. The resulting supernatant was analyzed spectrometrically at 750, 663, 645, and 630 nm. A trichromatic procedure was used to dif-

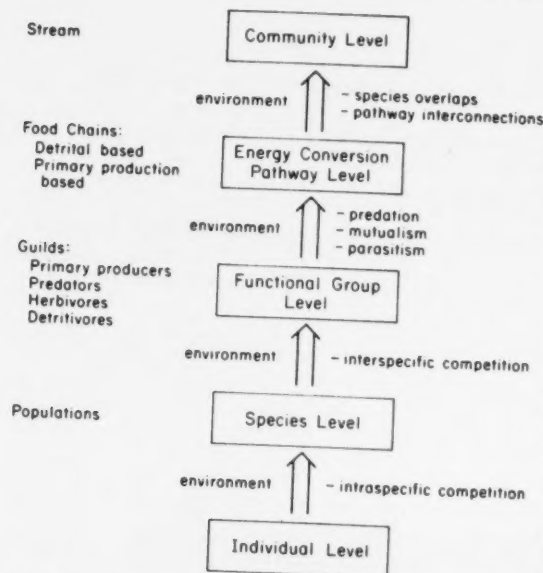


FIG. 2. Hierarchical levels of investigation of a stream community.

ferentiate between chlorophyll *a*, *b*, and *c* (Weber 1973). A monochromatic procedure was used for pheophytin determination (Weber 1973).

The biomass of the fungal flora in each stream channel was estimated on a relative basis from measurements of hyphal abundance on leaf material. A 5.0-g pack of dead alder leaves was prepared, incubated for 6 wk in the channel, removed, and cut into 0.5-cm discs. Ten of these discs were randomly selected, clarified with 5% KOH, hardened with 50% ethanol, and stained with lactophenol-trypan blue (Commonwealth Mycological Institute 1968). Using an ocular micrometer, we estimated the percent cover of hyphae on the leaf surface.

The composition of the fungal flora in each stream channel was estimated from an analysis of spores on the leaf packs. Twenty leaf discs were incubated overnight in filter-sterilized water to stimulate spore formation. These discs were then fixed in lactophenol. Microscopic identification ( $250\times$ , phase contrast) of spores on the periphery of each disc was used to estimate species abundances and calculate diversity (Peterson 1962, 1963a, b; Ingold 1942; Ingold and Cox 1957; Ranzoni 1953). This procedure is qualitative at best because of the uncertain relationships between hyphal abundance and spore formation.

The composition and biomass of the bacterial flora in each stream channel were estimated from a sequence of dilution plating, morphological sorting, and classification testing. A 1.0-mL blended aliquot from each stream sample was serially diluted, plated on PYG media, and incubated for 48 h at  $20^{\circ}\text{C}$ . Colonies were counted and typed according to morphological characteristics. When possible, each type was identified to genus by use of dichotomous testing procedures (Buchanan and Gibbons 1974; Shayegani et al. 1976; Steele 1979). Bacterial biomass was assumed directly proportional to cell density and expressed as cells/mL.

The determination of effects in the stream communities was made at the functional group level (Merritt and Cummins 1978) (Fig. 2). Each functional group is composed of a guild of species that perform a specific step in the community's energy processing pathway. The structure and function of each group is a result of its species composition and the interactions among its component species (e.g. competition) as well as the interactions among functional groups (i.e. predation, mutualism, parasitism, etc.). Analysis at this level of complexity is appropriate because it incorporates both direct toxic effects and indirect interaction effects, and the results are clearly transferable to the community level.

Each month, identified taxa were placed into their appropriate functional group(s): either invertebrate scrapers, invertebrate shredders, invertebrate predators, invertebrate collectors—gatherers—filterers, algal primary producers, bacterial decomposers, or fungal decomposers. Using diversity (Shannon's index,  $H'$ ) and biomass as the parameters of analysis, comparisons were made between treatment and control streams. Reduction in the biomass of a functional group is assumed indicative of a lowered processing rate for its particular energy processing step. Reduction in the diversity of a functional group is assumed indicative of reduced stability of the guild as a result of simplification of its underlying structure.

Throughout the study period, between-stream comparisons were made. The parameters of analysis were biomass and diversity of each of the functional groups. During the equilibration period, the equivalence of the 10 stream channels was assessed. For each test parameter, an analysis of variance and, subsequent, pairwise Student—Neuman—Keuls multiple-range tests were performed. During the treatment period, the existence of differences between the four treatment levels and the controls were assessed. For each test parameter, a Williams' test (Williams 1972) was performed, using average stream values for each of the two replicates per treatment level.

The experimental design used for the analysis of the data was a completely randomized design with subsampling (Steel and Torrie 1960). Sampling error determined from the within-stream samples was small compared to experimental error determined from variation between streams treated alike. This latter experimental error was the variance that was used to estimate the standard error of a treatment effect and was used in further analysis of the data using Student—Neuman—Keul's test and Williams' test (Williams 1972).

## Results

### SINGLE-SPECIES TOXICITY TESTS

The results from the acute (Table 3) and chronic (Table 4) toxicity tests reveal that insect and crustacean species were affected by diflubenzuron. Values for the LC<sub>50</sub> ranging from 1.0 to 1.8  $\mu\text{g/L}$  were determined for four insect and crustacean species. No acute response resulted for fish or molluscan species from exposure to the highest concentration tested (45  $\mu\text{g/L}$ ). A chronic effect level was obtained for only *Daphnia magna* (0.06  $\mu\text{g/L}$ ). No chronic effects were observed for fish, an alga, and the two mollusks tested at

TABLE 3. Acute toxicity test results with insecticide diflubenzuron.

| Test species                 | Test description                                                   | LC50 ( $\mu\text{g/L}$ ) | 95% confidence level |
|------------------------------|--------------------------------------------------------------------|--------------------------|----------------------|
| <i>Salmo gairdneri</i>       | Salmonid fish species — 96 h LC50                                  | No effect $\leq 45$      |                      |
| <i>Pimephales promelas</i>   | Non-salmonid fish species — 96 h LC50                              | No effect $\leq 45$      |                      |
| <i>Lebistes reticulatus</i>  | Non-salmonid fish species — 96 h LC50                              | No effect $\leq 45$      |                      |
| <i>Cricotopus</i> sp.        | Benthic insect species — 4th larval instar to pupae LC50           | 1.79                     | (1.48, 2.13)         |
| <i>Tanytarsus dissimilis</i> | Benthic detritivore insect species — 2nd to 3rd larval instar LC50 | 1.02                     | (0.56, 1.47)         |
| <i>Hyallela azteca</i>       | Benthic crustacean species — 96 h LC50                             | 1.84                     | (0.05, 3.71)         |
| <i>Daphnia magna</i>         | Planktonic crustacean species — 48 h LC50                          |                          |                      |
| Total hardness (mg/L)        |                                                                    |                          |                      |
|                              | 200                                                                | 4.42                     | (2.79, 8.63)         |
|                              | 100                                                                | 6.89                     | (3.98, 18.50)        |
|                              | 50                                                                 | 4.55                     | (2.58, 12.71)        |
| <i>Juga plicifera</i>        | Freshwater molluskan species — 96 h LC50                           | No effect $\leq 45$      |                      |
| <i>Physa</i> sp.             | Freshwater molluskan species — 96 h LC50                           | No effect $\leq 45$      |                      |

TABLE 4. Chronic toxicity test results with insecticide diflubenzuron.

| Test species                     | Test description                                                                        | LC50 ( $\mu\text{g/L}$ ) | 95% confidence level |
|----------------------------------|-----------------------------------------------------------------------------------------|--------------------------|----------------------|
| <i>Salmo gairdneri</i>           | Salmonid fish species — survival and growth in early life stages (30 d)                 | No effect $\leq 45$      |                      |
| <i>Pimephales promelas</i>       | Non-salmonid fish species — survival and growth in early life stages (30 d)             | No effect $\leq 45$      |                      |
| <i>Daphnia magna</i>             | Planktonic crustacean species — survival and reproduction in a full life cycle          | 0.062                    | (0.051, 0.071)       |
| <i>Selenastrum capricornutum</i> | Freshwater algal species — growth after a 120-h exposure                                | No effect $\leq 45$      |                      |
| <i>Juga plicifera</i>            | Freshwater molluskan species — survival, growth, and reproduction after a 3-wk exposure | No effect $\leq 45$      |                      |
| <i>Physa</i> sp.                 | Freshwater molluskan species — survival, growth, and reproduction after a 3-wk exposure | No effect $\leq 45$      |                      |

concentrations as high as 45  $\mu\text{g/L}$ . Using the "Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and its Uses," Federal Register 44FT15970 (March 15, 1979), a final acute toxicity value of 1.1  $\mu\text{g/L}$  and a final chronic toxicity value of 0.06  $\mu\text{g/L}$  were established.

#### LABORATORY STREAM COMMUNITIES

The taxa found in these laboratory stream systems and their functional group assignments are presented in Appendix 1. Organisms found in the stream samples were sorted using these lists of taxa and, subsequently, calculations of diversity and biomass for each functional group were made. Many genera were placed into more than one functional group because of the diversity of trophic function exhibited by different species within a single genus, as well as the variety of trophic functions exhibited by individual species. Table 5 shows the in-stream responses of the most common of these taxa to diflubenzuron exposure. These sensitivities coupled with the above functional assignments were used to elucidate

the mechanisms underlying alterations in each functional group.

In general, the invertebrate functional groups were the most severely impacted, experiencing rapid and permanent reductions in their biomass and diversity when exposed to  $\geq 1.0$   $\mu\text{g/L}$  of diflubenzuron (Fig. 3–6). These effects were the result of major reductions in many of their component aquatic insect populations, primarily among the mayflies, stone flies, and dipterans. Effects on the important caddis fly populations could not be determined as a result of their precipitous decline during the early stages of the experiment in both control and treatment streams. This situation was apparently due to a combination of adult emergence of some species and the failure of others to become established in these laboratory systems. In all groups, diversity showed an apparent graded dose-response relationship, with little or no reductions occurring at 0.1  $\mu\text{g/L}$ , intermediate reductions at 1.0  $\mu\text{g/L}$ , and maximal reductions at  $\geq 10.0$   $\mu\text{g/L}$ . The intermediate effects at 1.0  $\mu\text{g/L}$  were apparently due to the relative insensitivity of some dipteran taxa to 1.0  $\mu\text{g/L}$ , but their

TABLE 5. Effect of diflubenzuron on the most abundant stream invertebrates

|                              | Pretreatment density<br>(No./m <sup>2</sup> ) | Pretreatment biomass<br>(total mm/m <sup>3</sup> ) | Fate in control stream | Fate in treatment streams         |                                          |
|------------------------------|-----------------------------------------------|----------------------------------------------------|------------------------|-----------------------------------|------------------------------------------|
|                              |                                               |                                                    |                        | Concn. of diflubenzuron<br>(µg/L) | Effect                                   |
| <b>Ephemeroptera</b>         |                                               |                                                    |                        |                                   |                                          |
| <i>Baetis</i> spp.           | 36.0                                          | 57.2                                               | No change in 5 mo      | ≥1.0                              | Eliminated in 1 mo                       |
| <i>Epeoris</i> spp.          | 26.7                                          | 44.8                                               | Reduced ~50% in 5th mo | 0.1                               | Same as control                          |
| <i>Rithogena</i> spp.        | 16.4                                          | 90.6                                               | Reduced ~50% in 2nd mo | ≥10.0                             | Eliminated in 1 mo                       |
| <i>Paraleptophlebia</i> spp. | 30.2                                          | 91.2                                               | Eliminated in 4 mo     | ≤1.0                              | Same as control                          |
| <i>Ephemerella</i> spp.      | 46.4                                          | 94.6                                               | Reduced ~75% in 5th mo | ≥1.0                              | Eliminated in 1 mo                       |
|                              |                                               |                                                    | Reduced ~60% in 2nd mo | 0.1                               | Same as control                          |
|                              |                                               |                                                    | Reduced ~90% in 5th mo | ≥1.0                              | Eliminated in 1 mo                       |
|                              |                                               |                                                    |                        | 0.1                               | Same as control                          |
| <b>Plecoptera</b>            |                                               |                                                    |                        |                                   |                                          |
| <i>Peltoperla</i> spp.       | 1.8                                           | 3.0                                                | Eliminated in 4 mo     | ≥1.0                              | Eliminated in 1 mo                       |
| <i>Pteronarcys</i> spp.      | 0.9                                           | 3.3                                                | Eliminated in 4 mo     | 0.1                               | Same as control                          |
| <i>Nemoura</i> spp.          | 16.4                                          | 53.6                                               | Reduced ~50% in 2nd mo | ≥1.0                              | Eliminated in 1 mo                       |
| <i>Acroneuria</i> spp.       | 5.0                                           | 33.4                                               | Eliminated in 5 mo     | 0.1                               | Same as control                          |
|                              |                                               |                                                    | No change in 5 mo      | ≥1.0                              | Reduced ~90% in 3rd mo                   |
| <i>Hesperoperla</i> spp.     | 3.4                                           | 26.5                                               | No change in 5 mo      | 0.1                               | Eliminated in 4 mo                       |
|                              |                                               |                                                    |                        | ≥1.0                              | Same as control                          |
| <i>Isoperla</i> spp.         | 0.6                                           | 1.7                                                | No change in 5 mo      | 0.1                               | Reduced ~50% in 3rd mo                   |
| <i>Alloperla</i> spp.        | 13.0                                          | 48.6                                               | Reduced ~70% in 3rd mo | ≥1.0                              | Eliminated in 5 mo                       |
|                              |                                               |                                                    |                        | 0.1                               | Same as control                          |
|                              |                                               |                                                    |                        | ≥1.0                              | Eliminated in 1 mo                       |
|                              |                                               |                                                    |                        | 0.1                               | Same as control                          |
|                              |                                               |                                                    |                        | ≥1.0                              | Reduced ~60% in 1st mo                   |
|                              |                                               |                                                    |                        | 0.1                               | Eliminated in 3 mo                       |
|                              |                                               |                                                    |                        | 0.1                               | Same as control                          |
| <b>Diptera</b>               |                                               |                                                    |                        |                                   |                                          |
| Chironomini                  | 27.6                                          | 163.6                                              | No change in 5 mo      | ≥10.0                             | Reduced ~10–75% in 1st–4th mo            |
|                              |                                               |                                                    |                        |                                   | Eliminated in 5 mo                       |
| Tanytarsini                  | 18.4                                          | 42.4                                               | No change in 5 mo      | ≤1.0                              | Same as control                          |
|                              |                                               |                                                    |                        | ≥10.0                             | Reduced ~50–95% in 1st–4th mo            |
| Tanypodinae                  | 17.0                                          | 54.4                                               | No change in 5 mo      | ≤1.0                              | Eliminated in 5 mo                       |
|                              |                                               |                                                    |                        | ≥10.0                             | Same as control                          |
| Orthocladinae                | 37.4                                          | 87.8                                               | Reduced ~90% in 5th mo | ≤1.0                              | Reduced ~60–95% in 2nd–4th mo            |
|                              |                                               |                                                    |                        | ≥10.0                             | Eliminated in 5 mo                       |
|                              |                                               |                                                    |                        |                                   | Same as control                          |
|                              |                                               |                                                    |                        |                                   | Reduced ~30–75% in 1st–4th mo            |
|                              |                                               |                                                    |                        |                                   | Eliminated in 5 mo                       |
|                              |                                               |                                                    |                        | 1.0                               | Reduced ~50–90% in 2nd–5th mo            |
|                              |                                               |                                                    |                        | 0.1                               | Same as control                          |
| <b>Tricoptera</b>            |                                               |                                                    |                        |                                   |                                          |
| <i>Rhydcophila</i> spp.      | 5.6                                           | 32.8                                               | Reduced ~50% in 3rd mo | ≤50.0                             | Not significantly different from control |
| <i>Hydropsyche</i> spp.      | 2.5                                           | 15.6                                               | Eliminated in 4 mo     | ≤50.0                             | Not significantly different from control |
| <i>Micrasema</i> spp.        | 1.0                                           | 2.3                                                | Eliminated in 3 mo     | ≤50.0                             | Not significantly different from control |
|                              |                                               |                                                    | Eliminated in 5 mo     | ≤50.0                             | Not significantly different from control |
| <b>Coleoptera—Elmidae</b>    |                                               |                                                    |                        |                                   |                                          |
| <i>Heterolimnius</i> spp.    | 5.2                                           | 15.7                                               | No change in 5 mo      | ≤50.0                             | Same as control                          |
| <i>Optioservus</i> spp.      | 20.7                                          | 61.8                                               | No change in 5 mo      | ≤50.0                             | Same as control                          |
| <i>Zaitzevia</i> spp.        | 1.7                                           | 5.0                                                | No change in 5 mo      | ≤50.0                             | Same as control                          |
| <i>Narpus</i> spp.           | 1.5                                           | 4.9                                                | No change in 5 mo      | ≤50.0                             | Same as control                          |

TABLE 5 (Concluded)

|                                | Pretreatment density (No./m <sup>2</sup> ) | Pretreatment biomass (total mm/m <sup>2</sup> ) | Fate in control stream        | Fate in treatment streams       |                                                              |
|--------------------------------|--------------------------------------------|-------------------------------------------------|-------------------------------|---------------------------------|--------------------------------------------------------------|
|                                |                                            |                                                 |                               | Concn. of diflubenzuron (µg/L.) | Effect                                                       |
| Oligochaeta                    | 52.2                                       | 92.3                                            | Increased ~200% in 2nd-5th mo | ≥10.0                           | Increased ~2000% in 2nd and 3rd mo<br>Reduced ~95% in 4th mo |
| Gastropoda<br><i>Juga</i> spp. | 20.9                                       | 134.9                                           | No change in 5 mo             | ≤50.0                           | Same as control                                              |

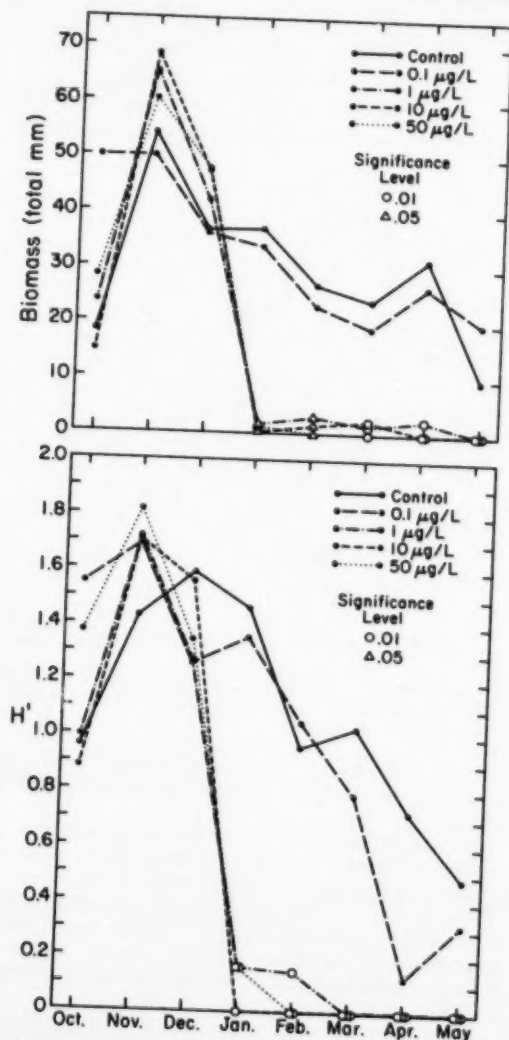


FIG. 3. Effects of diflubenzuron on the biomass (total cumulative length of organisms/m<sup>2</sup>) and the diversity ( $H'$ ) of the invertebrate shredder functional group.

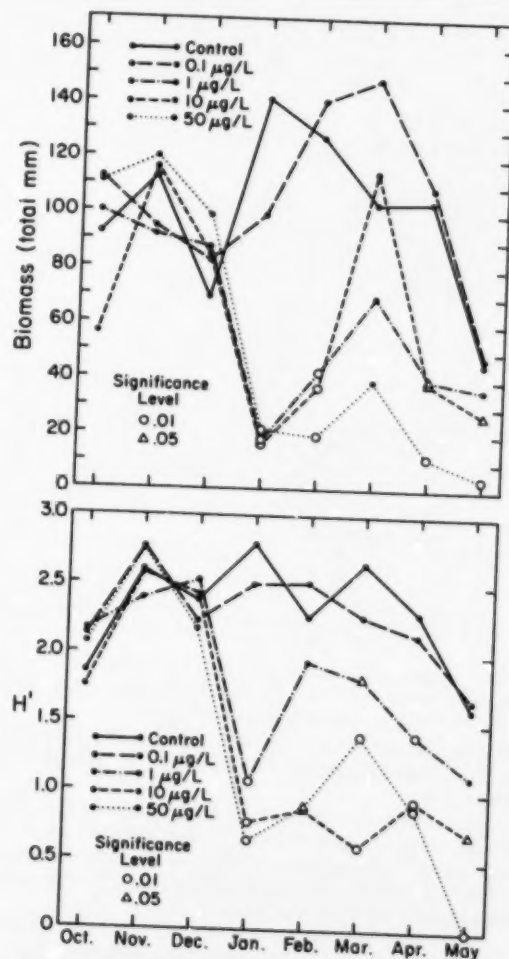


FIG. 4. Effects of diflubenzuron on the biomass (total cumulative length of organisms/m<sup>2</sup>) and the diversity ( $H'$ ) of the invertebrate scraper functional group.

elimination at the higher concentrations (Table 5).

This general scenario held for all of the invertebrate functional groups. However, because of differences in their

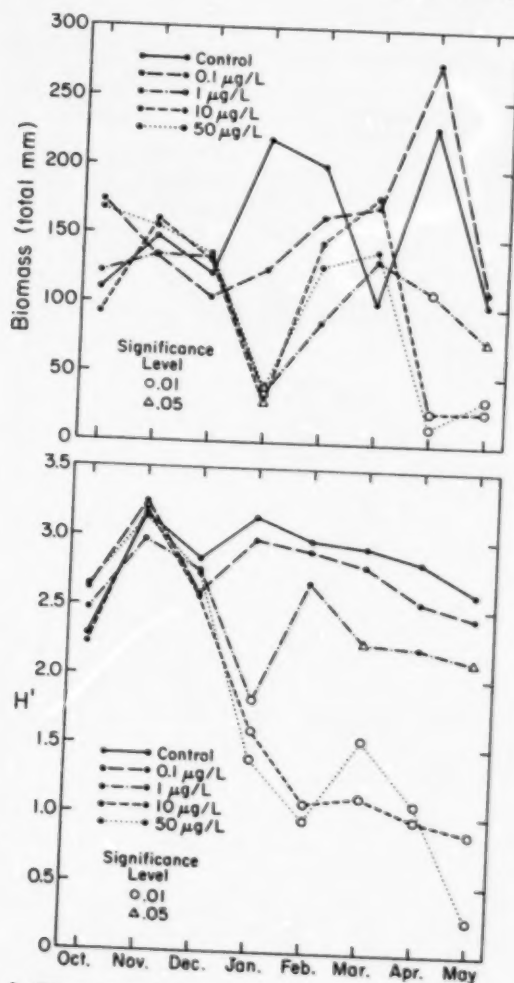


FIG. 5. Effects of diflubenzuron on the biomass (total cumulative length of organisms/m<sup>2</sup>) and the diversity ( $H'$ ) of the invertebrate collector-gatherer-filterer functional group.

species compositions (i.e. the relative abundance of dipterans in the functional group), variations between groups were apparent.

The shredder functional group (Fig. 3) was taxonomically simple, being composed mainly of mayflies and stone flies, and showed rapid and persistent effects. The graded diversity effect was not very distinct perhaps, as a result of the small dipteran component of this group.

The scraper functional group (Fig. 4) was more diverse, being composed of mayflies, stone flies, dipterans, beetles, and snails, and showed rapid, but less-persistent effects. The losses among the insect taxa were partially offset during January and February by temporary increases in the apparently insensitive snail, *Juga plicifera*, population. The riffle beetles were also insensitive and maintained their densities throughout the treatment period. The striking decline in diver-

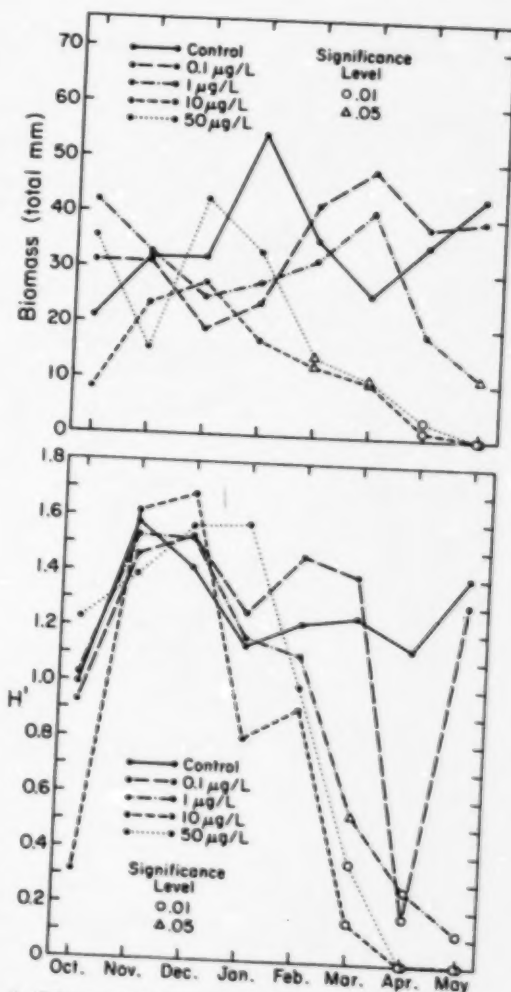


FIG. 6. Effects of diflubenzuron on the biomass (total cumulative length of organisms/m<sup>2</sup>) and the diversity ( $H'$ ) of the invertebrate predator functional group.

sity was a graded response with increasing concentration levels, primarily the result of reductions among the dipteran species.

The collector-gatherer-filterer functional group (Fig. 5), composed primarily of mayflies, stone flies, dipterans, beetles, and oligochaetes, showed rapid but discontinuous effects. In this case, the losses among the insect taxa were temporarily offset during January and February by increases in the insensitive oligochaete population. Again, because of the large dipteran component, the graded diversity response was clear.

The predator functional group (Fig. 6), being composed mainly of stone flies, dipterans, and beetles, showed slow but continuous effects. The gradual nature of these effects was the result of differences in sensitivity within the predominant

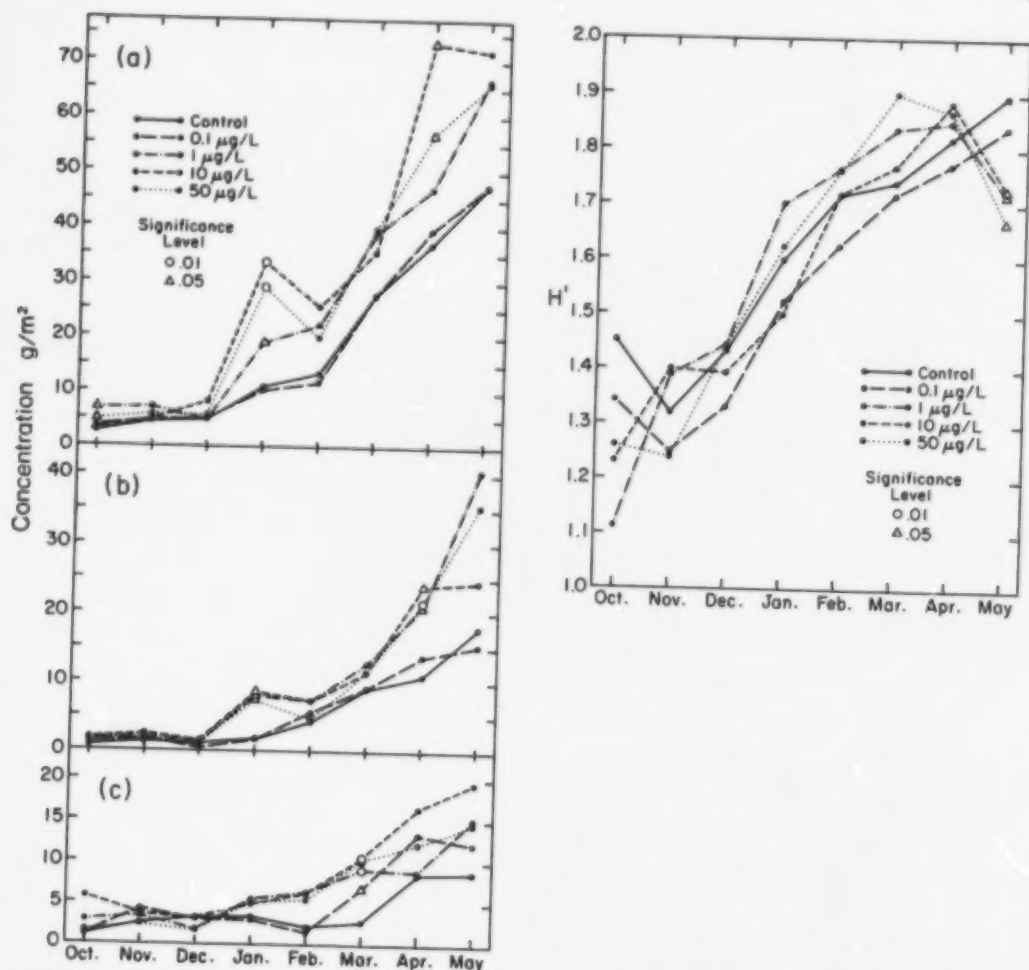


FIG. 7. Effects of diflubenzuron on the biomass (total cumulative length of organisms/m<sup>2</sup>) and diversity of the algal primary producer functional group. Biomass is expressed as concentrations of (a) chlorophyll a, (b) chlorophyll c, and (c) pheophytin. Diversity ( $H'$ ) was calculated using abundances of diatom species only.

stone fly component. Some stone flies (i.e. *Pteronarcys*, *Peltoperla*, *Nemoura*, and *Isoperla*) were rapidly eliminated, whereas others (i.e. *Acroneura*, *Hesperoperla*, and *Alloperla*) required several months of treatment before elimination occurred. The genera which survived the longest also had the longest instar periods. This is not surprising, because diflubenzuron has its major effect on chitin synthesis which occurs during molts between larval instars. This functional group also shows the graded diversity response, as a result of its large dipteran component.

The algal, fungal, and bacterial functional groups (Fig. 7-9) were also impacted by the addition of diflubenzuron. In general, these groups exhibited variable and transient alterations in biomass and diversity; algae and bacteria were affected at 1.0 µg/L, and the fungi were apparently affected by as little as 0.1 µg/L.

The algal primary producer functional group (Fig. 7) experienced rapid and persistent increases in biomass and delayed reductions in diversity associated with  $\geq 1.0$  µg/L of diflubenzuron. Immediately following equilibration, all streams contained (in order of relative abundance as indicated in parentheses): *Achnanthes lanceolata* (0.38), *Navicula* sp. (0.30), *Cocconeis placentula* (0.22), *Nitzschia* sp. (0.02), *Gomphonema accuminatum* var. *coronata* (0.02), and *Rhoicosphenia curvata* (0.02). Following the start of treatment, the streams receiving  $\geq 1.0$  µg/L of diflubenzuron experienced increased densities of all taxa, plus the early appearance of *Melosira varians* and *Synedra* sp. (appearing after 2 mo of treatment, compared to 4 mo in the control); effectively raising the biomass and diversity of these streams. Toward the end of the treatment period, algal density remained high in the streams containing  $\geq 1.0$  µg/L; however,

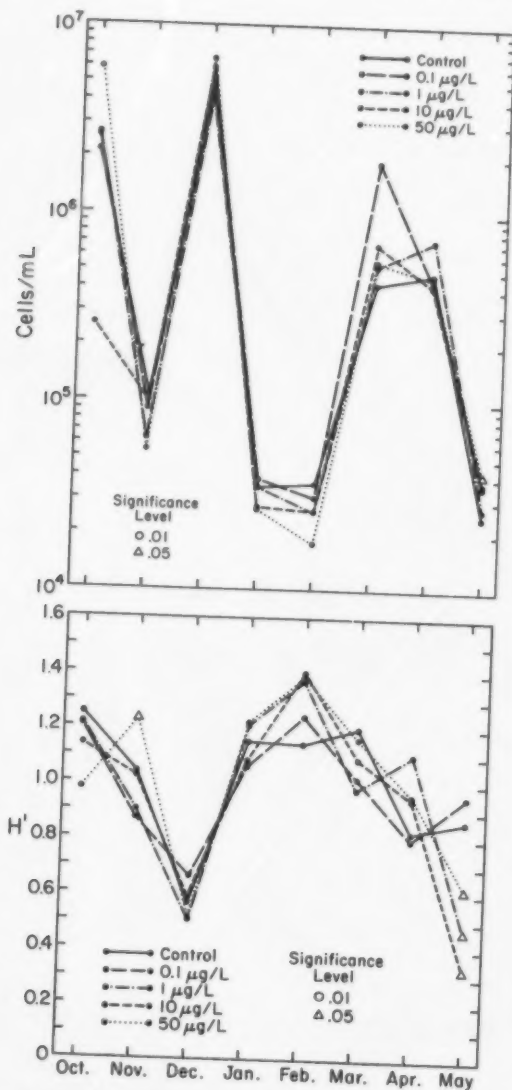


FIG. 8. Effects of diflubenzuron on the biomass (total cumulative length of organisms/ $\text{m}^2$ ) and the diversity ( $H'$ ) of the bacterial decomposer functional group.

*M. varians* and *Synedra* sp. became dominant, comprising 27% and 10%, respectively, of the overall algal abundance. Therefore, biomass remained elevated, but diversity declined.

The bacterial decomposer functional group (Fig. 8) experienced delayed reductions in diversity associated with exposure to  $\geq 1.0 \mu\text{g/L}$  of diflubenzuron; biomass was apparently unaffected at all treatment levels. Throughout the equilibration and treatment periods, all streams were qualitatively similar; *Pseudomonas* (0.38) and *Flavobacteria* (0.33) were most abundant, *Acinetobacter* (0.07), *Aeromonas* (0.06), and

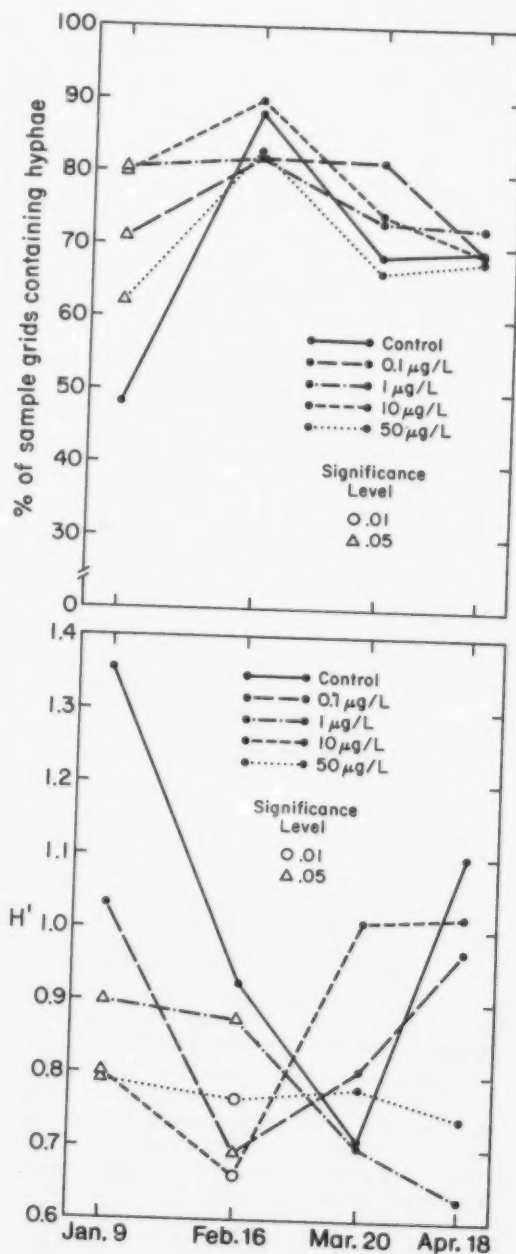


FIG. 9. Effects of diflubenzuron on the biomass (relative abundance of hyphae) and the diversity ( $H'$ , using spore abundances) of the fungal decomposer functional group at four sampling periods in 1980.

*Azotobacter* (0.05) were common, but less abundant. During the last treatment month, the streams contained  $\geq 1.0 \mu\text{g/L}$  and experienced shifts in the relative abundances of the

TABLE 6. Water quality characteristics — monthly averages (SD)

| Month | T<br>(°C)     | pH           | D.O.<br>(mg/L) | Hardness<br>(mg/L as<br>CaCO <sub>3</sub> equiv.) | Alkalinity<br>(mg/L as<br>CaCO <sub>3</sub> ) | NO <sub>3</sub> <sup>-</sup> -NO <sub>2</sub> <sup>-</sup><br>(mg/L) | NH <sub>3</sub><br>(mg/L) | Total<br>N<br>(mg/L) | Total<br>PO <sub>4</sub> <sup>-</sup><br>(mg/L) | O-PO <sub>4</sub> <sup>-</sup><br>(mg/L) |
|-------|---------------|--------------|----------------|---------------------------------------------------|-----------------------------------------------|----------------------------------------------------------------------|---------------------------|----------------------|-------------------------------------------------|------------------------------------------|
| Sept. | 14.2<br>(1.0) | 7.0<br>(0.2) | 9.9<br>(0.2)   | 28.5<br>(9.0)                                     | 2.4<br>(0.1)                                  | 0.148<br>(0.011)                                                     | 0.005<br>(0.003)          | 0.057<br>(0.047)     | 0.111<br>(0.015)                                | 0.051<br>(0.002)                         |
| Oct.  | 13.0<br>(1.3) | 7.0<br>(0.1) | 10.3<br>(0.2)  | 26.0<br>(3.8)                                     | 2.5<br>(0.1)                                  | 0.080<br>(0.019)                                                     | 0.007<br>(0.004)          | 0.040<br>(0.037)     | 0.087<br>(0.005)                                | 0.030<br>(0.006)                         |
| Nov.  | 12.8<br>(1.7) | 6.9<br>(0.1) | 10.5<br>(0.6)  | 25.5<br>(5.6)                                     | 2.3<br>(0.2)                                  |                                                                      |                           |                      |                                                 |                                          |
| Dec.  | 11.2<br>(1.2) | 7.2<br>(0.2) | 11.2<br>(1.2)  | 39.0<br>(15.1)                                    | 3.2<br>(1.0)                                  | 0.359<br>(0.024)                                                     | 0.096<br>(0.015)          | 0.016<br>(0.026)     | 0.074<br>(0.094)                                | 0.029<br>(0.005)                         |
| Jan.  | 10.2<br>(0.9) | 7.1<br>(0.1) | 11.3<br>(0.4)  | 48.5<br>(23.0)                                    | 3.5<br>(1.6)                                  | 0.066<br>(0.007)                                                     | 0.030<br>(0.013)          | 0.115<br>(0.025)     | 0.085<br>(0.012)                                | 0.036<br>(0.003)                         |
| Feb.  | 10.8<br>(0.6) | 7.1<br>(0.1) | 10.9<br>(0.3)  | 57.5<br>(4.4)                                     | 4.6<br>(0.4)                                  |                                                                      |                           |                      |                                                 |                                          |
| Mar.  | 11.2<br>(0.8) | 7.2<br>(0.0) | 10.8<br>(0.3)  | 60.8<br>(19.7)                                    | 4.9<br>(0.5)                                  | 0.510<br>(0.180)                                                     | 0.028<br>(0.033)          | 0.234<br>(0.261)     | 0.081<br>(0.185)                                | 0.014<br>(0.002)                         |
| Apr.  | 12.0<br>(0.9) | 7.2<br>(0.0) | 10.5<br>(0.2)  | 41.8<br>(6.0)                                     | 3.6<br>(0.4)                                  | 0.503<br>(0.008)                                                     | 0.014<br>(0.017)          | 0.131<br>(0.056)     | 0.035<br>(0.010)                                | 0.027<br>(0.007)                         |

TABLE 7. Measured concentrations (µg/L) of diflubenzuron in each stream channel, presented as monthly means ± SE.

| Nominal<br>Concn.<br>(µg/L) | Measured mean concentrations (µg/L) ± SD |              |             |             |            |
|-----------------------------|------------------------------------------|--------------|-------------|-------------|------------|
|                             | Dec.                                     | Jan.         | Feb.        | March       | April      |
| 0                           | <0.5                                     | <0.5         | <0.5        | <0.5        | <0.5       |
| 0                           | <0.5                                     | <0.5         | <0.5        | <0.5        | <0.5       |
| 0.1                         | <0.5                                     | <0.5         | <0.5        | <0.5        | <0.5       |
| 0.1                         | <0.5                                     | <0.5         | <0.5        | <0.5        | <0.5       |
| 1.0                         | 0.95±0.07                                | 0.93±0.15    | 1.05±0.21   | 0.84±0.15   | 0.89±0.02  |
| 1.0                         | 1.07±0.05                                | 1.10±0.04    | 1.02±0.02   | 0.81±0.09   | 0.83±0.09  |
| 10.0                        | 10.51±0.78                               | 9.06±1.30    | 11.28±1.33  | 9.51±0.98   | 10.49±0.84 |
| 10.0                        | 9.47±0.70                                | 9.80±0.47    | 12.65±0.65  | 9.03±0.89   | 10.50±0.91 |
| 50.0                        | 49.38±6.46                               | 59.25 (8.17) | 59.67±18.12 | 79.0±14.07  | 90.0±5.48  |
| 50.0                        | 55.13±6.35                               | 61.75±7.85   | 56.0±16.31  | 83.05±10.83 | 92.33±5.79 |

dominant species; *Pseudomonas* (0.83) increased and *Flavobacteria* (0.10) and *Acinetobacter* (0.01) decreased. These alterations reduced species evenness and, therefore, reduced diversity. Because these alterations were observed at the conclusion of this study, their permanence could not be determined.

The results relating to the fungal decomposer functional group were inconclusive. Reductions in diversity and elevations in biomass (Fig. 9) were apparently associated with exposure to  $\geq 1.0$  µg/L of diflubenzuron. However, these effects were transient and variable in nature, lasting only a couple of months and showing no clear dose-response relationship. Also, because no fungal determinations were available until the start of diflubenzuron addition, pretreatment equivalence between streams could only be assumed.

#### STREAM CHEMISTRY

The water quality characteristics of the stream channels

(Tables 6 and 7) are recorded as monthly mean values. For physical and inorganic parameters (Table 6), no statistically significant differences were apparent between any of the stream channels. Therefore, these parameters are reported as averages over all channels. The diflubenzuron concentrations (Table 7) purposely differ between treatment levels and are reported as monthly averages for each of the stream channels. Actual concentrations in the 0.1-µg/L treatment level and the control streams are not reported because the detection limit of the analytical method was 0.5 µg/L.

#### Discussion

The results of this study indicate that a standard set of single-species toxicity tests were adequate to predict the concentrations of the insecticide diflubenzuron that affected the structure and function of these complex laboratory stream communities. The calculated final chronic toxicity value of 0.06 µg/L is approximately an order of magnitude lower than

the minimum toxicant concentrations that affected the biomass and/or diversity of all stream functional groups.

This correspondence supports the assumption that the standard set of test species is representative of the range of sensitivities found in natural assemblages of species, at least at the genus level. Because the identification of many stream organisms could not be made to the species level, a critical test of the relative sensitivities of all species could not be made. Nevertheless, for all cases in which species identifications were available, no underprotected species were found.

Our study also indicates that the single-species tests did not predict the exact nature of all the effects observed in the stream communities. Effects which resulted from direct lethality to constituent species were well predicted. For example, reductions in the diversity and biomass of the invertebrate functional groups were fully expected, as a result of the high sensitivity of insects and crustaceans in the single-species tests. However, effects which resulted from altered interspecies interactions could not be predicted, without a priori knowledge of the trophic dynamics of the system. For example, the observed alterations in the biomass and diversity of the algal functional group were not expected; diflubenzuron produced no effect in the *Selenastrum* single-species test. The increase in algal biomass was an indirect effect, probably as a result of decreased predation by the impacted invertebrate herbivores. The subsequent decrease in algal diversity was apparently the result of increased competition for limited attachment sites among the enlarged algal populations. The best competitors under crowded conditions have been shown to be *M. varians* and *Synedra* sp. (Patrick 1977), and it was these populations which increased at the expense of less-adapted species. Without the knowledge that primary producers were initially herbivore limited and that release from this herbivore control would produce competition for space, these changes could not be accurately forecast.

This lack of correspondence between expected and observed community responses casts doubt upon the assumption that interactions need not be considered as a result of their relative unimportance or insensitivity. Apparent alterations in predator-prey and competition interactions produced significant shifts in the structure of the algal functional group. These interaction effects were approximately as sensitive as the direct lethal effects observed in the invertebrate functional groups, being exhibited at the 1.0- $\mu\text{g/L}$  level.

The conclusions we draw from this study are probably conservative. Because of the limited duration of the study and the relative simplicity of these laboratory stream communities in relation to natural communities, the likelihood of finding differences between the single-species predictions and the community level responses should be minimized. These stream microcosms have a finite lifetime of ~8 mo, and only effects which appear within that time frame can be assessed. For many important stream organisms, this time period represents only a portion of one generation. Therefore, neither reproductive effects nor long-term interaction effects, which may take several generations to become apparent, can be evaluated. These stream microcosms are physically nearly homogeneous and, consequently, have less-diverse community structure than is found in natural systems. Therefore, some very sensitive species and/or interactions may be miss-

ing, and the effects on them will not be assessed.

A determination of the generality of these results will require additional research using a variety of chemicals and community types. Because of differences in modes of action and degrees of specificity, various classes of toxicants when added to the same community may differentially affect groups of species and types of interactions. As a result of differences in species composition and the relative importance that interactions play in community structure, various community types may respond differently to the same toxic substance. An overall assessment of many community types and toxicant classes will be necessary to establish the robustness of the assumptions underlying the use of single-species toxicity testing. This is one such study.

### Acknowledgments

Special thanks are extended to Rosemarie Booze, Dena Gadomski, Floyd Hutchins, Phillip McKinney, Teri Poulis, and Dale West for their invaluable assistance in establishing, sampling, and analyzing the results from these stream systems. Appreciation is also directed to the entire staff at Western Fish Toxicology Station for their conceptual and physical help.

- ANDROS, J. D., AND R. R. GARTON. 1980. Acute lethality of copper, cadmium, and zinc to northern squawfish. *Trans. Am. Fish. Soc.* 109: 235-238.
- ASTM. 1980. Standard E 72980. Practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103, USA.
- BUCHANAN, F. E., AND N. E. GIBBONS. 1974. *Bergey's Manual of determinative bacteriology*, 8th ed. Williams and Wilkins Company, Baltimore, MD. 1268 p.
- CAIRNS, J. 1980. Beyond single species toxicity testing. *Mar. Environ. Res.* 3: 157-159.
- COMMONWEALTH MYCOLOGICAL INSTITUTE. 1968. *Plant pathologist's pocketbook*. Commonwealth Mycological Institute, Kew, England. 267 p.
- CORLEY, C., R. W. MILLER, AND K. R. HILL. 1974. Determination of N(4-chlorophenyl)-N'(2,6-difluorobenzoyl)-urea milk by high speed liquid chromatography. *J. Assoc. Off. Anal. Chem.* 57: 1269-1271.
- EISELE, P. J., AND R. HARTUNG. 1976. The effects of methoxychlor on riffle invertebrate populations and communities. *Trans. Am. Fish. Soc.* 105: 628-633.
- GARTON, R. R. 1980. A simple continuous-flow toxicant delivery system. *Water Res.* 14: 227-230.
- GECKLER, J. R., W. B. HORNING, T. M. NEHEISEL, Q. H. PICKERING, AND E. L. ROBINSON. 1976. Validity of laboratory tests for predicting copper toxicity in streams. *Ecol. Res. Ser.*, U.S. Environ. Prot. Agency, EPA-600/3-76-116. Duluth, MN.
- GIBSON, J. R. 1981. Safety margins: classical mammalian approaches and practical aspects of their present use in aquatic toxicology, p. 10-13. *In* D. R. Branson and K. L. Dickson [ed.] *Fourth Conference on Aquatic Toxicology and Hazard Assessment*, ASTM STP737. Am. Soc. Test. Mater. Proc.
- HEATH, R. T. 1979. Holistic study of an aquatic microcosm: theoretical and practical implications. *Intern. J. Environ. Stud.* 13: 87-93.
- HITCHCOCK, S. W. 1965. Field and laboratory studies of DDT and aquatic insects. *Conn. Agric. Exp. Stn. Bull.* (New Haven) 668: 1-32.
- INGOLD, C. T. 1942. Aquatic Hyphomycetes of decaying alder leaves. *Trans. Brit. Mycol. Soc.* 25: 339-417.

- INGOLD, C. T., AND V. J. COX. 1957. *Heliscus stellatus* sp. nov. an aquatic hyphomycete. Trans. Brit. Mycol. Soc. 40: 155-158.
- MERRITT, R. W., AND K. W. CUMMINS. 1978. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company, Dubuque, IA. 441 p.
- MOUNT, D. I. 1979. Adequacy of laboratory data for protecting aquatic communities, p. 112-118. In K. L. Dickson, A. W. Maki, and J. Cairns [ed.] Analyzing the Hazard Evaluation Process. Am. Fish. Soc.
1981. Margins of safety for aquatic communities, p. 5-9. In D. R. Branson and K. L. Dickson [ed.] Fourth Conference on Aquatic Toxicology and Hazard Assessment, ASTM STP737. Am. Soc. Test. Mater. Proc.
- N.R.C. 1981. Review for effects of chemicals on ecosystems: a report by the committee to review methods for ecotoxicity. National Academy Press, Washington, DC.
- NEBEKER, A. V. 1973. Temperature requirements and life cycle of the midge *Tanytarsus dissimilis* (Diptera: Chironomidae). J. Kansas Entomol. Soc. 46: 160-165.
- OEHLER, D. D., AND G. M. HOLMAN. 1975. Residue determination of Thompson-Hayward 6040 in bovine manure by high performance liquid chromatography. J. Agric. Food Chem. 23: 590-591.
- PATRICK, R. 1977. Ecology of freshwater diatoms and diatom communities, p. 234-242. In D. Werner [ed.] The biology of diatoms. Botanical monographs, Vol. 13. Blackwell Scientific Publications, Oxford, England.
- PETERSEN, R. H. 1962. Aquatic Hyphomycetes from North America. I. Aleurisporae (Part I), and key to the genera. Mycologia 54: 117-151.
- 1963a. Aquatic Hyphomycetes from North America II. Aleurisporae (Part II), and Blastosporae. Mycologia 55: 18-29.
- 1963b. Aquatic Hyphomycetes from North America. III. Phialosporae and miscellaneous species. Mycologia 55: 570-581.
- RANZONI, F. V. 1953. The aquatic Hyphomycetes of California. Farlowia 4: 353-398.
- REISA, J. J. 1981. Margins of safety in the assessment of aquatic hazards of chemicals—some regulatory viewpoints, p. 15-27. In D. R. Branson and K. L. Dickson [ed.] Fourth Conference on Aquatic Toxicology and Hazard Assessment: ASTM STP737. Am. Soc. Test. Mater. Proc.
- SAMUELSON, D. F. 1976. Water quality: western fish toxicology station and western Oregon rivers. Ecol. Res. Ser., U.S. Environ. Prot. Agency, EPA-600/3-76-077, Duluth, MN.
- SHAYEGANI, M., A. M. LEE, AND L. M. PARSON. 1976. A scheme for the identification of non-fermentative gram-negative bacteria. Health Lab. Sci. 14: 83-90.
- STEELE, M. B. 1979. Identification of non-fermentative Gram-negative bacilli by conventional biochemicals and dichotomous scheme. Wilmington Medical Center, Delaware Division Microbiology, Wilmington, DE.
- STEELE, R., AND J. H. TORRIE. 1960. Principles and procedures of statistics: with special reference to the biological sciences. McGraw-Hill Book Company, Inc. New York, NY. 481 p.
- U.S.E.P.A. 1975. Methods for acute toxicity tests with fish, macro-invertebrates, and amphibians. Ecol. Res. Ser., U.S. Environ. Prot. Agency, EPA-660/3-75-009, Corvallis, OR.
- Methods for ecological toxicology: a critical review of laboratory multispecies tests. Office of Toxic Substances, U.S. Environ. Prot. Agency, EPA-560/11-80-026, Washington, DC.
- WEBER, C.I. 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. Environ. Monit. Ser., U.S. Environ. Prot. Agency, EPA-670/4-73-001, Cincinnati, OH.
- WEISS, C. M. 1976. Field evaluation of the algal assay procedure on surface waters of North Carolina, p. 29-76. In E. J. Middlebrooks, D. H. Falkenberg, and T. E. Maloney [ed.] Biostimulation and nutrient assessment, Ann Arbor Science, Ann Arbor, MI.
- WILLIAMS, D. A. 1972. The comparison of several dose levels with a zero dose control. Biometrics 28: 519-531.

## Appendix

### 1. Invertebrate scrapers

#### Ephemeroptera

*Baetis*, *Epeorus*, *Cinygmula*, *Rithrogena*, *Paraleptophlebia*, *Ephemerella*, *Stenoma*

#### Plecoptera

*Pteronarcella*, *Brachyptera*, *Paraperla*

#### Tricoptera

*Neophylax*, *Glossosoma*, *Dicosmoecus*, *Anagapetus*, *Leptoceridae*, *Hesperophylax*, *Ecclisomyia*

#### Diptera

*Pericoma*, *Blepharicera*

#### Coleoptera

*Optioservus*, *Cleptelmis*, *Zaitzevia*, *Heterlimnius*, *Narpus*, *Ampumixis*

#### Mollusca

*Juga plicifera*

### 2. Invertebrate shredders

#### Ephemeroptera

*Paraleptophlebia*, *Ephemerella*

#### Plecoptera

*Pteronarcys*, *Pteronarcella*, *Nemoura*, *Leuctra*, *Peltoperla*, *Capnia*

#### Tricoptera

*Onocosmoecus*, *Hydatophylax*, *Micrasema*, *Lepidostoma*, *Hesperophylax*

#### Diptera

*Tipula*

#### Coleoptera

*Lara*

### 3. Invertebrate collectors—gatherers—filterers

#### Ephemeroptera

*Ameletus*, *Baetis*, *Epeorus*, *Cinygmula*, *Rithrogena*, *Paraleptophlebia*, *Ephemerella*, *Stenonema*

#### Plecoptera

*Isoperla*, *Alloperla*, *Peltoperla*

#### Tricoptera

*Hydatophylax*, *Wormaldia*, *Hydropsyche*, *Polycentropus*, *Micrasema*, *Hesperophylax*, *Ecclisomyia*

#### Diptera

*Antocha*, *Prosimulium*, *Simulium*, *Pericoma*, *Dixa*, *Tipula*, *Chironomini*, *Tanytarsini*, *Corynoneurini*, *Orthocladini*

#### Coleoptera

*Optioservus*, *Oreodytes*, *Zaitzevia*, *Heterlimnius*, *Narpus*, *Ampumixis*

#### Miscellaneous

Ostracoda, Copepoda, Cladocera, Oligochaeta, Nematoda

### 4. Invertebrate predators

#### Plecoptera

- Pteronarcella*, *Acroneuria*, *Hesperoperla*, *Isoperla*,  
*Alloperla*, *Isogenus*, *Paraperla*
- Tricoptera  
*Rhyacophila*, *Polycentropus*
- Diptera  
*Bessia*, *Hemerodromia*, *Pedicia*  
 Pentaneurini, Ceratopogoninae, Empididae, Tany-  
 podini, Tabanidae
- Coleoptera  
*Oreodytes*, *Hydroporus*  
 Carabidae
- Odonata  
 Octogomphus
- Miscellaneous  
*Scalis* (neuroptera), *Hydracuriana* (mite)  
 Hydrozoa
5. Algal primary producers  
*Achnanthes lanceolata*, *Navicula* sp., *Cocconeis*  
*placentula*, *Nitzschia* sp., *Gomphonema acuminatum*  
 var. *coronata*, *Rhoicosphenia curvata*, *Melosira*  
*varians*, *Synedra* sp., *Meridion circulare*, *Surirella* sp.,  
*Fragilaria* sp., *Cymbella minuta*, *Cymbella sinuata*,  
*Cyclotella* sp., *Frustulia rhomboides*, *Epithemia tur-*  
*gidida* var. *Westermanni*, *Hantzschia* sp., *Eunotia ar-*  
*cus*, *Pinnularia subcapitata* var. *pavastriata*, *Pin-*  
*nuh.ria mesolepta* var. *mesolepta*
6. Fungal decomposers  
*Heliscus stellatus*, *Articulospora tetraccladia*, *Tetra-*  
*chaetum elegantus*, *Heliscus longibranchus*, *Clavariop-*  
*sis aquatica*, *Lunulospora curvula*, *Anguillospora pseu-*  
*dolongisma*, *Tetraccladium marchalianum*
7. Bacterial decomposers  
*Pseudomonas*, *Flavobacteria*, *Acinetobacter*, *Aero-*  
*monas*, *Azotobacter*, *Enterobacter*, *Moraxella*, *Micro-*  
*coccus*, *Lactobacillus*, *Alcaligenes*, *Xanothomonas*,  
*Beijerinckia*, *Azomonas*, *Vibrio* *Beggiatoaceae*